Effect of high temperature on haemolymph sugar levels in three selected silkworm races

Firdose Ahmad MALIK, Y. Srinivasa REDDY*

(Department of Studies in Sericultural Science, University of Mysore, Manasagangotri, Mysore 570006, India)

Abstract: Two bivoltine races *viz*. NB4D2 and CSR2 acclimatized to temperate climates, and one multivoltine race *viz*., Pure Mysore (PM) acclimatized to tropical climates were exposed to two selected high temperatures of 32℃ and 36℃ during 5th instar larval and pupal stages. The tropical multivoltine PM showed less larval and pupal mortality than temperature tolerant bivoltine NB4D2 and temperature sensitive bivoltine CSR2. Haemolymph trehalose levels were relatively higher, and glucose level and trehalase activity lower during moult. At 32℃ and 36℃, haemolymph sugar level and trehalase activity showed only a marginal increase during larval moult. Moulted larvae showed a significant drop in haemolymph trehalose level and increase in trehalase activity and glucose levels. Also, the moulted larvae showed a further decrease in trehalose levels and increase in glucose level and trehalase activity when exposed to higher temperature. Feeding larvae showed a significant drop in glucose level and increase in trehalose level in haemolymph at 25 ± 1℃. At 32℃ and 36℃, haemolymph glucose and trehalose levels and trehalase activity increased in feeding larvae in PM and NB4D2 but decreased in CSR2. Spinning larvae showed a significant decrease in haemolymph glucose level and trehalase activity and marginal decrease in trehalose level. At higher temperatures, an increase in blood sugar level and trehalase activity was observed in spinning larvae in temperature tolerant PM and NB4D2 and decrease in temperature sensitive CSR2. Haemolymph sugar level and trehalase activity increased during pupal development in all the three races. At the two higher temperatures, the haemolymph sugar level and trehalase activity increased in PM. But, in NB4D2 the increases in haemolymph sugar level and trehalase activity in pupae at 36℃ were less than those at 32℃. In CSR2, an increase in haemolymph glucose level was observed at 32℃ but haemolymph glucose decreased to the level lower than the control when the ambient temperature of the pupae was raised to 36℃. But, haemolymph trehalose level and activity decreased when the pupae of CSR2 were held at higher temperatures, the decrease being more at 36℃ than at 32℃. Acclimation to high temperature in the mulberry silkworm, *Bombyx mori* thus depended upon the race and developmental stage in the life cycle and was manifested by the changes in haemolymph sugar level and trehalase activity.

Key words: *Bombyx mori*; haemolymph; glucose; trehalose; trehalase; temperature tolerance; acclimation

1 INTRODUCTION

Terrestrial insects are often exposed to constant high ambient temperatures in their natural environment. Even domesticated economic insects like the mulberry silkworm, *Bombyx mori* L., are exposed to extremes of ambient temperatures as they are distributed across different latitudes. Exposures to the prevailing high or low ambient temperatures lead to the development of voltinism in *B. mori*. Bivoltine races with embryonic diapause evolved in temperate climates as multivoltine races acclimated to high ambient temperatures of tropical climatic conditions. Both bivoltine and multivoltine races are reared in large quantity in tropical Indian subcontinent in all seasons of the year. As the two voltine races are adapted to contrasting temperature regimes, significant differences in their adaptation to high temperature in summer season are noticed. The traditional multivoltine race, Pure Mysore (PM) is well acclimated to high temperature as it has been bred and reared continuously for over 200 years in tropical Indian climates. NB4D2 evolved as a line from a double hybrid cross of Japanese parents [(Kokko (Seihaku) × (N124 (J124)] is considered as a temperature tolerant bivoltine race since its introduction in the year 1972. CSR2, an inbred line derived from a cross between Japanese parents (Shurei × Shogetsu) spins oval cocoons which is a Chinese character in bivoltine silkworm races. CSR2 is less temperature tolerant than NB4D2 and introduced to tropical climates more recently in 1998. The period of exposure to hot climates

* Author for correspondence: Prof. Y. Srinivasa Reddy, DOS in Sericultural Science, University of Mysore, Manasagangotri, Mysore 570006, Tel.: 0091-9448293656; Fax: 0091821-2419335; E-mail: ysr-yre@yahoo.com

Received: 2008-04-29; Accepted: 2008-10-23

thus plays a more significant role than the thermal history of parents in high temperature adaptation of race. A significant difference in the larval and pupal mortality among the three races was observed during rearing in summer seasons of the year. There are practically no studies on the role of the factors responsible for the differences in the temperature tolerance observed in the three races.

Thermal stress is a complex syndrome caused by increase in core body temperature (T_b) by high ambient temperatures. A poikilothermic insect can not control T_b like a homeotherm. The insects therefore have both external and internal protective mechanisms which contribute to the temperature tolerance observed in the organism. The relative roles of the external and internal protective mechanisms are different at different developmental stages in the life cycle of the insect. The external protective mechanisms considerably reduce the permeation of outer ambient temperatures to interior and affect the body temperature. The most important among the external protective mechanisms in insects are outer epidermal layers like cuticle and epicuticular fat blooms. The impenetrability of the cuticle depends on the thickness and organization of the cuticular layers which is different at different stages of larval growth like moult, feeding and spinning and pupal development. It becomes necessary therefore to strengthen internal protective mechanisms at developmental stages when the functional ability of the external protective mechanisms is temporarily weakened to accommodate the demands of progress of development. One such internal mechanism that provides protection against rising T_b is changing levels of haemolymph sugars.

Haemolymph sugars play a significant role in minimizing the damaging effects of thermal stress which is a complex process involving a number of physiological and biochemical changes and membrane functions (Shinozaki and Dennis, 2003). Insect haemolymph has a large pool of sugars from which all the tissues of the insect body withdraw fuel resources to meet their metabolic needs (Satake *et al*., 2000). Glucose and trehalose are the principal sugar components in insect haemolymph. Glucose which is a reducing sugar is readily absorbed by all the tissues to meet their immediate metabolic needs. The metabolic rate of the tissues increases by an increase in core body temperature leading to higher rates of utilization of haemolymph glucose levels. The changes in haemolymph glucose levels therefore reflect the metabolic needs of the animal exposed to high temperature. Trehalose is a non reducing disaccharide present in the haemolymph of many insects (Becker *et al*., 1996). It is considered as a major transport and storage sugar available for utilization by the tissues with surface trehalase activity which develops at specific stages of growth in certain selected tissues like ovary during active vitellogenesis (in 4th and 5th instar larvae and pupae), and silk gland during silk biosynthesis in late 5th instar stage of larval development. Other tissues utilize trehalose by its hydrolysis through soluble and surface trehalase activity in the haemolymph. Trehalose is a multifunctional molecule, and diverse functions like structural support, transport role (Takayama and Armstrong, 1976), signaling, and protection of membranes and proteins against heat or cold have been attributed to the sugar. The organizations of cuticular layers and changes in haemolymph sugar levels at selected developmental stages have been known in some insects but the relative roles played by them in the temperature tolerance have not been evaluated. It is therefore felt necessary to study the temperature tolerance in three selected races of the mulberry silkworm, *B. mori* and results examined in relation to the relative roles of external protective mechanisms which are significantly different at different larval and pupal stages and haemolymph sugars at those stages of development.

2 MATERIALS AND METHODS

2.1 Animals

The silkworm breeds namely, Pure Mysore, NB4D2 and CSR2 were utilized in the present investigation to represent a tropical multivoltine, less productive bivoltine and highly productive bivoltine respectively. Disease free layings (DFLs) of the three races were brushed and reared as per the standard rearing technique of Dandin *et al*. (2003). Fresh mulberry leaves of V-1 variety were used for feeding the larvae.

2.2 Temperature treatments and observations on larval and pupal mortality

The optimum temperature recommended for rearing 5th instar larvae is 25 ± 1℃ in all seasons. But, the ambient temperatures in tropical countries range from 30 – 35℃ in summer season and hence 32℃ and 36℃ were selected for high temperature treatment. The larvae were transferred one day before 4th moult into the chambers with three temperature zones *viz*., 25 ± 1℃ (control), 32℃ and 36℃ controlled with different thermostats representing optimal, high and very high temperatures respectively. The relative humidity (RH) was held constant at 70% ± 5% at all the three temperatures. The cocoons were cut open on the 3rd day after spinning and naked pupae were held in temperature regimes as above and haemolymph was used

for sugar estimations. The larval and pupal morality was observed till the end of the larval and pupal growth period and percent mortality was calculated in the treated batches at the two selected temperatures.

2.3 Haemolymph collection

Haemolymph was collected in a pre-chilled test tube containing a few crystals of thiourea by cutting the first proleg of larva. Haemolymph from pupae was obtained by piercing a sharp sterilized syringe needle into the first abdominal segment and applying gentle pressure on the thorax and abdomen. The haemolymph was collected from the larvae in 4th moult, after moult, feeding and spinning stages, and pupae at different developmental stages in control and temperature treated batches of the three races. The haemolymph was centrifuged at 3 000 g for 10 min at 4℃ and the supernatant was used in the enzyme assay and sugar estimations.

2.4 Estimation of glucose

Glucose level in the haemolymph was estimated by Folin-Wu method (1976). 100 μL of haemolymph supernatant and 7.9 mL of distilled water were taken in glass test tube and mixed with 1 mL of 10% sodium tungstate solution and 1 mL of 0.66N H_2SO_4. The mixture was filtered and 2 mL of haemolymph filtrate was transferred to a Folin-Wu-tube and 2 mL of alkaline copper reagent was added and boiled for 8 min. After cooling, 2 mL of phosphomolybdic acid was added to each test tube. After 1 min, distilled water was added till 12.5 mL mark. The contents of each tube were then mixed by repeated inversion and the extinction coefficient was observed at 420 nm in a spectrophotometer.

2.5 Estimation of trehalose (O-α-D-glucopyranosyl [1→1] - α-D- glucopyranoside)

Trehalose level in the haemolymph was estimated by the method of Steele *et al*. (1988). To 100 μL of haemolymph, 5 mL of anthrone reagent was added and boiled for 10 minutes. The contents in the tubes were cooled and the colour developed was read against the blank at 620 nm. Trehalose levels were determined by deducting glucose from total anthrone positive substances as trehalose and glucose constitute the two predominant haemolymph sugars in insects.

2.6 Estimation of trehalase activity (α, α-trehalose glucohydrolase; EC 3.2.1.28)

Trehalase activity was assayed by the method of Friedman (1966). 300 μL of haemolymph (enzyme solution) was mixed with 10 mmol trehalose and 60 mmol citrate buffer of pH 5.6 and incubated at 32℃ for 15 min. The reaction was stopped by addition of 1 mL of Ba $(OH)_2$ (0.5 mol) and 1 mL of $ZnSO_4$ (0.5 mol). The solution was made up to 10 mL with distilled water. The mixture was centrifuged and aliquots of the supernatant were used for the estimation of glucose. The activity of trehalase was expressed as mg glucose released per mL per hour.

2.7 Statistical analysis

Analysis of variance was used to test the significance of difference between the mean values of six independent observations of glucose and trehalose level, and trehalase activity of the haemolymph of silkworm larvae and pupae. Tukeys (1953) multiple comparison tests were used to find significance of difference between the races, treatments and different developmental stages. Differences were considered significant at $P < 0.05$.

3 RESULTS

3.1 Larval and pupal mortality

Larval morality upon exposure to high temperature was observed in all the three races both at 32℃ and 36℃ (Fig. 1). The larval mortality was relatively less at 32℃ than at 36℃ in all the three races. Among the races, PM showed a relatively less mortality at both the temperatures than the two bivoltine races of NB4D2 and CSR2. Between the two bivoltine races, CSR2 showed a relatively higher larval mortality than NB4D2 at both temperatures.

Pupal mortality was observed at both 32℃ and 36℃ in all the three races, the mortality being higher at 36℃ than at 32℃ in each race. Among the races, pupal mortality was significantly higher in bivoltine races than PM at higher temperatures. Between the two bivoltine races, the pupal mortality was relatively higher in CSR2 than NB4D2 when held at 32℃ and 36℃. The pupal mortality was significantly lower than the larval mortality within each race at the two higher temperatures.

3.2 Haemolymph glucose levels

The levels of trehalose and glucose and the trehalase activity in the haemolymph showed significant changes at different stages of larval and pupal development in all the three races of the silkworm (Figs. 2 – 4).

The glucose level were relatively lower during moult but in moulted larvae before first feeding, the glucose levels showed a significant increase in all the three races (Fig. 2). In the feeding larvae, a decrease in haemolymph glucose was observed in all the three races. The spinning larvae showed a further decrease in haemolymph glucose content. The glucose levels improved significantly through different stages of pupal development namely prepupae, pupae and pharate adult in all the three races.

When 5th instar larvae were exposed to higher

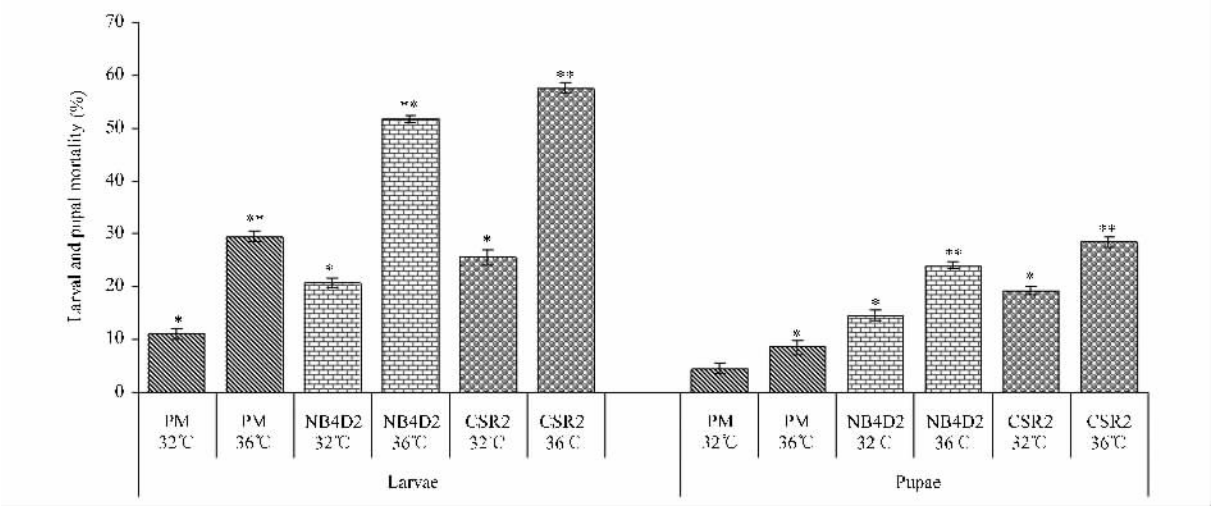

Fig. 1 Effect of high temperatures on larval and pupal mortality in different breeds of the silkworm, *Bombyx mori* L.
Each value is the mean ± *SD* of 6 separate observations. Asterisks indicate statistically significant differences between the means (**: $P < 0.01$; *: $P < 0.05$). The same for the following figures.

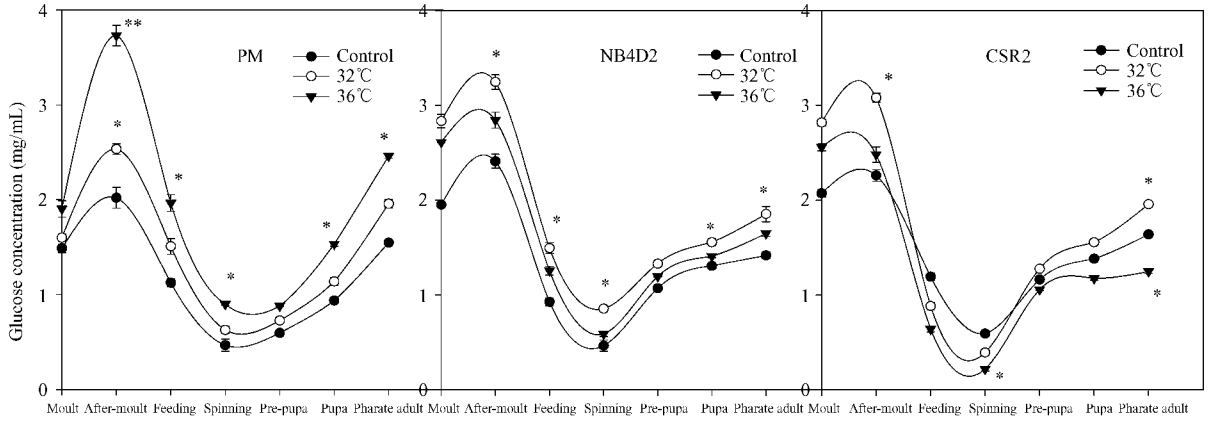

Fig. 2 Glucose levels in the haemolymph when larvae and pupae of three different races of the silkworm, *Bombyx mori* L. at different developmental stages were exposed to two selected high temperatures

temperature, the glucose levels increased significantly in larvae after moult than in moult, the relative increase being higher at 36℃ than at 32℃ in PM. Higher haemolymph glucose levels were observed in other stages of larval and pupal development in PM. In NB4D2, the increase observed at 36℃ was relatively lower than the increase observed at 32℃ in all the larval and pupal stages. In CSR2, the changes observed in haemolymph glucose levels of different larval and pupal stages depended on the limits of the temperature tolerance of the stage. Higher temperature caused an insignificant increase in haemolymph glucose level when the larvae of CSR2 were in moult. In moulted larvae of CSR2, the increase in haemolymph glucose level was more at 32℃ than at 36℃. In feeding and spinning larvae of CSR2, haemolymph glucose levels dropped to levels less than the control, the decrease being more 36℃ than at 32℃. The haemolymph glucose levels increased when the pupae of PM were held at higher temperatures of 32℃ and 36℃ but the increase was higher at 32℃ than 36℃ in NB4D2.

In CSR2, the haemolymph glucose levels were higher when the pupae were held at 32℃ but decrease to levels less than control at 36℃.

3.3 Haemolymph trehalose levels

Haemolymph trehalose levels in moulting larvae were relatively higher in the bivoltine races of NB4D2 and CSR2 than the multivoltine PM (Fig. 3). A significant drop in trehalose levels was observed in moulted larvae in all the three races. The haemolymph trehalose levels increased significantly in feeding and spinning larvae and an acute drop in haemolymph trehalose levels was observed in prepupae in all the three races. But the levels of trehalose improved significantly throughout the pupal stages.

Trehalose levels increased significantly when

moulted larvae were exposed to 32℃ in all the three races. Higher trehalose levels were observed in feeding and spinning larvae at 32℃ in PM and NB4D2. But, a decrease in trehalose levels was observed in feeding and spinning larvae of CSR2 at 32℃. At 36℃, trehalose levels showed higher increase in feeding and spinning larvae than at 32℃ in PM whereas in NB4D2 the increase was relatively less and in CSR2 a decrease in trehalose levels was noticed.

Trehalose levels in pupal stages increased at 32℃ in PM and NB4D2 whereas in CSR2, a decrease was observed. At 36℃, a significant increase in trehalose levels was observed in PM whereas in NB4D2 the increase was relatively less and in CSR2, a significant decrease was observed.

3.4 Haemolymph trehalase activity

Trehalase activity change at different stages of larval and pupal development in all the three races. The activity of the enzyme increased in moulted and feeding larvae but decreased in spinning larvae (Fig. 4). The trehalase activity showed a significant increase throughout the pupal stages in all the three races.

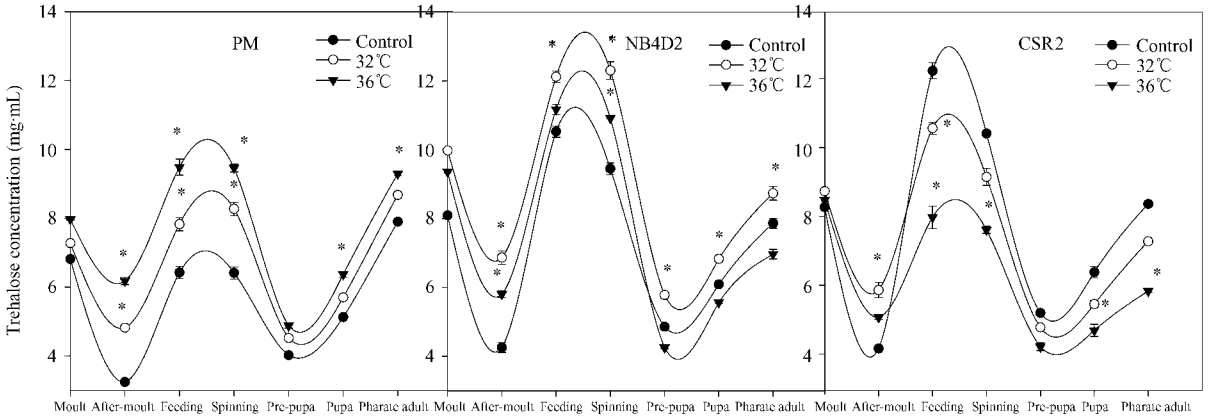

Fig. 3　Trehalose levels in the haemolymph when larvae and pupae of three different races of the silkworm, *Bombyx mori* L. at different developmental stages were exposed to two selected high temperatures

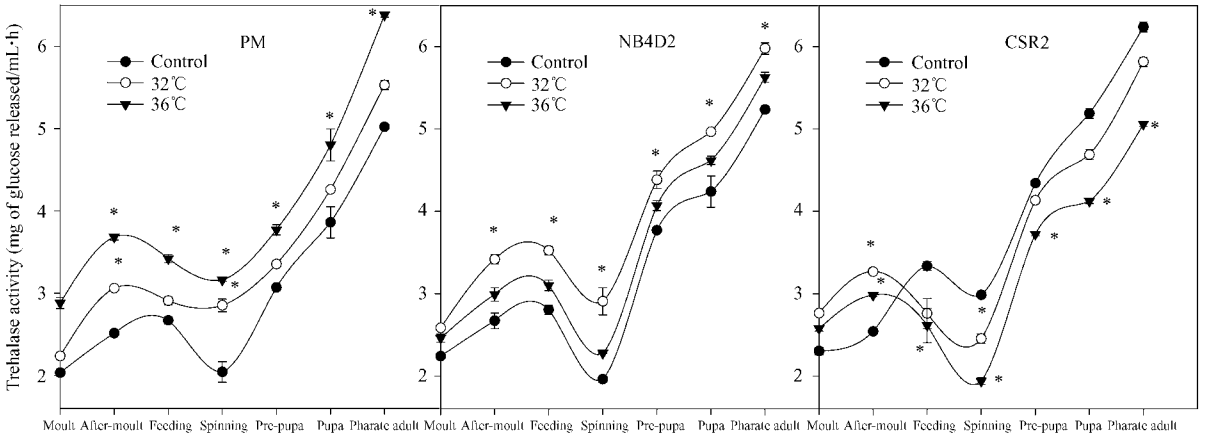

Fig. 4　Trehalase activity levels in the haemolymph when larvae and pupae of three different races of the silkworm, *Bombyx mori* L. at different delvelopmental stages were exposed to two selected high temperatures

At higher temperature, the haemolymph trehalase activity showed a significant increase in larvae which cleared 4th moult from the levels in moult. The increase was higher in moulted and feeding larvae at 36℃ than 32℃ in PM and 32℃ than at 36℃ in NB4D2. In CSR2, the trehalase activity showed more increase at 32℃ than at 36℃ in moulted larvae but in feeding and other larval and pupal stages the enzyme levels decrease which was more at 36℃ than at 32℃.

4 DISCUSSION

Environmental temperature played a significant role in the racial distribution of mulberry silkworm, *B. mori*. Temperature tolerance of the race depends on the ambient temperature to which the race has been acclimatized in its natural environment. The racial difference in the mortality of the larvae held at the two temperatures reflects the temperature range in which the

evolution of the race has taken place. The tropical multivoltine, PM showed the lowest larval and pupal mortality at higher temperature than the two bivoltine races. Among the bivoltine races, CSR2 which has been most recently introduced to tropical climates showed higher larval and pupal mortality than NB4D2. The pupal mortality was relatively lower than the larval mortality within each race, as the pupae are covered by hardened puparium which provides a better insulation to pupae inside against high ambient temperatures than the relatively thin layer of cuticle on the larval epidermis to the silkworm larva.

The levels of haemolymph sugars are maintained by a state of equilibrium between their release into haemolymph from the tissues like midgut and fat body on one hand and their metabolic utilization and other losses of the sugar on the other, and are determined by "the phase of the stadium", the stadium itself and the diet (Friedman, 1978). Hypoglycemia and hypertrehalosemia observed in larvae during moult reflects the metabolic state of the tissues till the old skin was shed completely. The larvae in moult will be inactive and stationary and will not feed during moulting. Non-epidermal tissues showed very little activity during moulting, and hence the glucose levels in haemolymph are significantly less on account of reduced metabolic needs of most of the tissues. The haemolymph glucose levels in moulted larvae even before first feeding showed an increase signifying resumption of normal activity of the tissues. Many tissues of the silkworm cannot store large quantity of glucose but utilize it as a source of energy for their metabolic activity. The tissues withdraw glucose from the haemolymph which is a large pool of glucose in proximity to the tissues. The levels of haemolymph glucose in feeding larvae are relatively low as excess glucose is utilized for glycogen synthesis in the fat body. Siegert (1987) reported a decrease in fat body glycogen by 20 mg in fifth instar larvae of *Manduca sexta* starved for 24 h. Low haemolymph glucose levels in feeding larvae provide a favorable gradient for glucose absorption from the lumen of the midgut. A significant decrease in haemolymph glucose levels in spinning larvae can be attributed to utilization of haemolymph glucose for muscular activity related to spinning. The haemolymph glucose levels increased in pupal stages as pupal development involves significant synthetic activity in relation to reorganization of larval tissues into imaginal structures presumably from fat body glycogenolysis.

Hypertrehalosemia in larvae at moult and highly significant decrease in haemolymph trehalose levels in moulted larvae suggest utilization of trehalose for chitin synthesis. 30% of the chitin is reabsorbed and new chitin synthesis is necessarily required during each moult for deposition on enlarged epidermal surface (Merzendorfer and Zimoch, 2003). Relatively greater decrease of haemolymph trehalose in PM could be related to initial lower trehalose levels at moult. Haemolymph trehalose levels increase significantly in feeding larvae and decrease during the end of the growth phase when silk biosynthesis is highest (data not shown). Further, the increase in trehalose level in feeding larvae in the three races correlate with the quantity of silk synthesized by their silk glands. Malik and Reddy (2007) reported that silk productivity, which is the quantity of silk synthesized as a function of 5th instar larval duration, is relatively higher in the two bivoltine races of CSR2 and NB4D2 than the multivoltine PM race. Thus, silk gland appears to use haemolymph trehalose as a source of energy for increased silk biosynthetic during 5th instar stage of larval development. The haemolymph trehalose content in the proximity of silk gland in *B. mori* increases with the growth of the silk gland and reaches maximum before spinning (Shimada *et al.*, 1980). Unni *et al.* (1997) reported increased trehalase activity in the silk gland, fat body and haemolymph at the beginning of spinning activity in the muga silkworm, *Antheraea assama*.

Trehalose was utilized as a source of energy for larval-pupal transformation more than for spinning activity as the drop in the haemolymph trehalose levels from spinning larvae to prepupae was greater than between feeding and spinning larvae. The trehalase activity of the silk gland were highest during spinning activity in the muga silkworm, *A. assama* (Unni *et al.*, 1997). A consistent increases in trehalose levels along with glucose levels in haemolymph during pupal development suggests increased demand of the fuel reserves for larval-pupal and pupal-imaginal transformation obviously by fat body glycogenolysis.

Trehalase activity and trehalose levels of haemolymph in 5th instar larvae are inversely related. An increase in trehalase activity and a drop in haemolymph trehalose levels were observed in moulted larvae. Increase in haemolymph trehalose levels in feeding larvae with an unchanged trehalase activity might be due to increased levels of its release from the fat body tissue into the haemolymph. A hypertrehalosemic principle secreted by neurosecretory cells of the brain increases haemolymph trehalose levels by fat body glycogenolysis (Steele, 1988). A significant drop in haemolymph trehalase activity and haemolymph trehalose levels in bivoltine races in spinning larvae suggests utilization of trehalose for spinning activity by surface trehalase activity of somatic tissues like the silk gland. Increased haemolymph trehalose levels despite increases observed in haemolymph trehalase activity during the course of

pupal development might be the result of relatively greater synthesis and release of trehalose from the fat body into the haemolymph to meet the increased demands of energy resources for the pupal development. A significant decrease in fat body glycogen was observed during larval-pupal transition and pupal development.

Tolerance to constant high temperature exposure depends on the collateral functional efficiency of external and internal protective mechanisms at different stages of growth and development in insect life cycle. The silkworm in moult is normally exposed to higher temperature and dry environment (Dandin *et al*., 2003). Tolerance of larvae in moult to higher temperature despite lower functional efficiency of transforming cuticular layer depends on temporary increase in the water content of haemolymph which prevents dehydration of tissues at higher ambient temperature. Ziegler *et al*. (2000) observed that the sudden increase in the haemolymph volume between late premoult and intramoult served to expand the cuticle during moult in the isopod, *Ligia pallasii*. Also, relatively higher trehalose content protects haemolymph and tissue proteins from damaging effects of high temperature. Moulting and feeding larvae had higher basal trehalose levels and hence the percent increases observed consequent to exposure to higher temperature were relatively less. Increase in haemolymph osmolarity on account of high trehalose levels protects the organism against dehydration stress at high ambient temperatures (Behm, 1997). The biological membranes are protected against desiccation by replacement of the water in lipid bilayer with trehalose keeping the lipid bilayers in crystalline state and preventing the transition to gel phase, with the consequent loss of structural and functional membrane integrity (Behm, 1997). Kaushik and Bhat (2003) reported an increase in transition temperature (Δ_{Tm}) of proteins in the presence of trehalose by increase in the surface tension of water, and having a large hydrated volume around proteins requires much higher temperatures for protein denaturation. Greater increase in haemolymph trehalose levels observed in multivoltine PM than in the two bivoltine races might be one of the physiological adaptations responsible for higher temperature tolerance observed in the race. When the nematode *Aphelenchus avenae* was slowly dehydrated, trehalose constituted 20% of its dry weight and a significant correlation between the ability of this organism to withstand dehydration and synthesis of trehalose was observed (Madin and Crowe, 1975; Womersley, 1981). When the larvae were exposed to high ambient temperatures of 32℃ and 36℃, greater mortality was observed in bivoltine races of NB4D2 and CSR2 than the multivoltine PM race despite greater trehalose level in bivoltine races. Kaushik and Bhat (2003) observed that the nature of the proteins also plays an important role in trehalose mediated thermal stress of the proteins. At lower temperatures, trehalose provides a preferential hydration of RNase A in cold adaptation whereas at high temperature trehalose binds weakly to RNase A (Kaushik and Bhat, 2003). Thus, proteins adapted to contrasting temperature regimes in the races of the two voltine groups might bind differently to trehalose. A stronger protein-trehalose interaction could be expected in temperature tolerant PM race exposed to high ambient temperatures than NB4D2 and least trehalose mediated protection in CSR2 race. Trehalose-mediated protection might be restricted to low temperature in the two bivoltine races of *B. mori* by virtue of their evolutionary episodes in regions of cold climates. An eight-fold increase in trehalose levels was observed in cold resistant *E. coli* when ambient temperatures were reduced from 37℃ to 16℃ (Kandror *et al*., 2002). Prolonged cold storage at 5℃ of *Nematodirus battus* eggs led to the accumulation of trehalose acting as a cryoprotectant enhancing the ability of the eggs to supercool (Behm, 1997). Non epidermal tissues will be inactive during moult and thus are affected only marginally by higher ambient temperatures requiring only a small rise in haemolymph sugar levels to meet the metabolic needs of the tissues at higher temperatures. On the other hand, moulted larvae respond more significantly by an increase in haemolymph sugar levels as well as trehalase activity. In the absence of feeding, the increase in haemolymph sugar levels in the moulted larvae could be attributed to their mobilization from fat body tissue. The feeding and spinning larvae showed higher haemolymph sugar levels and trehalase activity at higher temperature in temperature tolerant PM and NB4D2, whereas decrease in the levels was noticed in temperature sensitive CSR2 race. The falling haemolymph sugar levels at higher temperatures might be the result of failing physiological compensatory mechanisms leading to larval and pupal death. Lower levels of haemolymph sugar were observed at 36℃ in NB4D2 and at both 32℃ and 36℃ in CSR2. The haemolymph sugar level showed a linear increase at 32℃ and 36℃ in temperature tolerant PM indicating normal physiological response to high temperature. The small pool of haemolymph glucose responds readily than the larger pool of haemolymph trehalose to imposed thermal stress on the organism. It cannot be said whether the falling haemolymph sugar levels were the cause or the result of thermal injury of the organism. The haemolymph sugar level and trehalase activity were less affected in pupae exposed to higher temperatures as the hardened puparium prevents a synchronous rise in the body temperature in higher ambient temperature environment.

References

Becker A, Schl der P, Steele JE, Wegener G, 1996. The regulation of trehalose metabolism in insects. *Experientia*, 52(5): 433 – 439.

Behm CA, 1997. The Role of Trehalose in the Physiology of Nematodes. *International Journal for Parasitology*, 27: 215 – 229.

Dandin SB, Jayant Jayaswal, Giridhar K, 2003. Handbook of Sericulture Technologies. Central Silk Board, Bangalore, India. 195 – 205.

Folin A, Wu T, 1976. Determination of creatinine. In: Hawk HB, Oser BL, Summerrson WH eds. Practical Physiological Chemistry. Vol. 12. McGraw-Hill Pub. Co., New York. 1 052 – 1 054.

Friedman S, 1966. Trehalase from insects. Methods of Enzymology. Vol. VIII. Academic Press, New York. 600 – 420.

Friedman S, 1978. Metabolism of carbohydrates in insects. In: Florkin M, Scheer BT eds. The Chemical Zoology. Academic Press, New York. 5: 167 – 197.

Kandror O, Deleon A, Goldberg AL, 2002. Trehalose synthesis is induced upon exposure of *Escherichia coli* to cold and is essential for viability at low temperatures. *Proc. Natl. Acad. Sci. USA*, 99: 9 727 – 9 732.

Kaushik JK, Bhat R, 2003. Why is trehalose an exceptional protein stabilizer. *J. Biol. Chem.*, 278: 26 458 – 26 465.

Madin KAC, Crowe JH, 1975. Anhydobiosis in nematodes: Carbohydrate and lipid metabolism during dehydration. *J. Exp. Zool.*, 193: 335 – 342.

Malik FA, Reddy SY, 2007. Role of mulberry nutrition on manifestation of post cocoon characters of selected races of the silkworm, *Bombyx mori* L. *Sericologia*, 47: 63 – 76.

Merzendorfer H, Zimoch L, 2003. Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. *J. Exp. Biol*, 206: 4 393 – 4 412.

Satake S, Kawabe Y, Mizoguchi A, 2000. Carbohydrate metabolism during starvation in the silkworm *Bombyx mori*. *Arch. Insect Biochem. Physiol*. 44: 90 – 98.

Shimada S, Masaru K, Kamada A, Shojia A, 1980. Trehalose in the silk glands of the silkworm *Bombyx mori*. *Insect Biochem*, 10: 175 – 177.

Shinozaki K, Dennis ES, 2003. Cell signaling and gene regulation: global analyses of signal transduction and gene expression profiles. *Curr. Opin. Plant Biol.*, 6: 405 – 409.

Siegert KJ, 1987. Carbohydrate metabolism in starved fifth instar larvae of *Manduca sexta*. *Arch. Insect Biochem., Physiol.*, 4: 151 – 160.

Steele JE, 1988. Occurrence of a hyperglycaemic factor in the corpus cardiacum of an insect. *Nature*, 192: 680 – 681.

Takayama K, Armstrong EL, 1976. Isolation, characterization and function of 6-mycolyl-6 acetyltrehalose in the H37Rv strain of *Myocobacterium tuberculosis*. *Biochemistry*, 15: 441 – 447.

Tukey JW, 1953. The problem of multiple comparison. In: Snedecor GW ed. Statistical Methods. Iowa State College Press, Ames, Iowa.

Unni BG, Choudhury A, Khanikor D, Ghosh AC, 1997. Studies on trehalase and trehalose in the fatbody, haemolymph, silkgland and head region of the muga silkworm, *Antheraea assama*, during the spinning period. *Sericologia*, 37: 31 – 35.

Womersley C, 1981. Biochemical and physiological aspects of anhydobiosis. *Comp. Biochem. Physiol.*, 70: 669 – 272.

Ziegler A, Grospietsch T, Carefoot TH, Danko JP, Zimmer M, Zerbst-Boroffka I, Pennings SC, 2000. Haemolymph ion composition and volume changes in the supralittoral isopod, *Ligia pallasii* Brandt, during molt. *J. Comp. Physiol.* [*B*], 170: 329 – 336.

高温对家蚕三品系血淋巴中糖水平的影响

Firdose Ahmad MALIK, Y. Srinivasa REDDY*

(Department of Studies in Sericultural Science, University of Mysore, Manasagangotri, Mysore 570006, India)

摘要: 家蚕 *Bombyx mori* 的两个二化性品系热耐受型 NB4D2 和热敏感型 CSR2 均适合于温带气候,而多化性的 PM (Pure Mysore) 品系适合于热带气候,将这 3 种品系 5 龄幼虫分别置于 32℃和 36℃的高温下,观察高温对其 5 龄幼虫至蛹期血淋巴中糖含量及海藻糖酶活性的影响。结果表明: PM 幼虫和蛹的死亡率均小于 NB4D2 和 CSR2。在蜕皮期间血淋巴海藻糖水平较高,而葡萄糖水平及海藻糖酶活性较低。32℃和 36℃的高温下,幼虫蜕皮期间血淋巴中糖含量及海藻糖酶活性仅在其各自的水平上表现为小幅度的增加。蜕皮后幼虫血淋巴中海藻糖含量显著下降,而葡萄糖含量和海藻糖酶活性显著上升。在较高温度下,蜕皮后幼虫血淋巴中海藻糖含量下降幅度更大,而葡萄糖含量及海藻糖酶活性上升水平也更加显著。25 ± 1℃下取食幼虫血淋巴中葡萄糖含量显著下降,海藻糖含量显著上升; 32℃和 36℃下 PM 和 NB4D2 取食幼虫血淋巴葡萄糖和海藻糖含量以及海藻糖酶活性增加,而 CSR_2 均减少或降低。吐丝幼虫血淋巴中葡萄糖含量及海藻糖酶活性显著下降,海藻糖小幅度下降。而在较高温度下,耐热型 PM 和 NB4D2 吐丝家蚕血淋巴糖含量含量和海藻糖酶活性明显增加,而热敏感型 CSR2 的则明显下降。这 3 种品系蛹发育期的血淋巴糖含量及海藻糖酶活性均下降。在两较高温度下, PM 蛹期血淋巴糖和海藻糖酶活性增加,而 NB4D2 36℃时增加幅度小于 32℃时。对于 CSR2, 32℃时观察到其血淋巴葡萄糖含量增加,但当环境温度增加到 36℃时其血淋巴葡萄糖含量降至正常水平下。然而,当 CSR2 的蛹置于 32℃和 36℃时血淋巴海藻糖含量及其酶活性下降,且 36℃时下降幅度更大。因此,桑蚕对高温的适应取决于家蚕的品系及发育阶段,并可通过其血淋巴糖及海藻糖酶活性水平进行验证。

关键词: 家蚕; 血淋巴; 葡萄糖; 海藻糖; 海藻糖酶; 耐温性; 适应

中图分类号: Q966 **文献标识码**: A **文章编号**: 0454-6296(2008)11-1113-08

(责任编辑: 袁德成)